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<b>(21) International Application Number:</b> PCT/US96/12488 <b>(22) International Filing Date:</b> 30 July 1996 (30.07.96)  <b>(30) Priority Data:</b> 60/001,711      31 July 1995 (31.07.95)      US  <b>(71) Applicant (for all designated States except US):</b> THE ORAL- IFE GROUP, INC. [CA/CA]; 4th floor, 401 The West Mall, Toronto, Ontario M9C 5J5 (CA).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> KERSCHENSTEINER, Daniel, A. [US/US]; 121 Reville Road, Wayne, PA 19087- 5835 (US).  <b>(74) Agent:</b> MONACO, Daniel, A.; Seidel, Gonda, Lavorgan & Monaco, P.C., Suite 1800, Two Penn Center Plaza, Philadelphia, PA 19102 (US).		<b>(81) Designated States:</b> CA, GB, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> A COLORIMETRIC METHOD OF DETECTING THIOL OR MERCAPTAN COMPOUNDS AND ITS USE FOR ORAL MALODOR DETERMINATION		
<b>(57) Abstract</b>  The invention relates to a method for detecting the presence of thiol, mercaptans, sulfhydryl or volatile sulfur compounds in a sample and to reagents and reaction mixtures which can be used in detecting such compounds. More particularly it relates to colloidal metal sol suspensions which have a flocculated state visually distinguishable from a monodisperse suspended state and can be used in detecting thiol compounds. The tensioned or sensitized state of colloidal metal sol suspensions are prepared and subsequently exposed to a sample which may contain thiol compounds. The presence of such compounds can be determined by the colour change of the sol. The reagents and reaction mixtures are used in the diagnosis of halitosis, as halitosis is related to the presence of thiol and volatile sulfur compounds in the breath sample of an individual. The invention also relates to halitosis diagnostic kits comprising a reagent or reaction mixture of the invention and a blow tube.		

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**A Colorimetric Method of Detecting Thiol or Mercaptan Compounds and its  
Use for Oral Malodor Determination**

**Field of the Invention**

5 This invention relates to a method for detecting thiol, mercaptan, sulfhydryl compounds or volatile sulfur compounds in a sample. It also relates to a method for detecting halitosis related to the presence of such compounds in a breath sample of a patient. In different aspects, the invention relates to compositions and kits which can be used in detecting such compounds

**Background of the Invention**

10 Halitosis, defined as unpleasant oral odor, has become a health concern among the general public. The main source of halitosis is the presence of volatile sulfur compounds such as hydrogen sulfide and methyl mercaptan with some dimethyl mercaptans, in the mouth air. Septic and unsanitary oral conditions are often the major cause of halitosis. Halitosis is generally diagnosed by organoleptic  
15 examination, which is the sensed occurrence of a foul smell by another individual, and/or by gas chromatographic analysis of volatile sulfur compounds present in the mouth air. These techniques require use of costly equipment and timely analysis and do not afford individual privacy during the diagnostic process.

20 Since halitosis can be quite embarrassing for those afflicted, there is a need to detect halitosis in a self-administered, easy, timely and cost effective manner, especially, for instance, before an important meeting or social engagement.

Colloidal metal sols, such as colloidal gold and colloidal silver are known. They are generally prepared by the method of Frens G. "Controlled Nucleation for the Regulation of Particle Size in Monodisperse Gold Suspensions" *Nat. Phys. Sci.*  
25 241:20, (1973) and are principally produced having a particle size ranging from 10

to 120 nanometers (nm). Colours of these suspensions range in appearance from orange to red to violet to purple. Typical concentrations of these suspensions range from 0.01 to 0.05 weight percent.

- Colloidal gold sol suspensions are anionic and unstable. A small amount of ions in the form of monovalent, divalent, or higher cations cause the particles to flocculate, clump together or come out of suspension, as a result of an unstabling influence on the suspension. Examples of flocculating salts are sodium chloride, calcium (II) chloride and aluminum (III) chloride. Acids such as hydrochloric acid or citric acid, causing a pH drop of the suspension below pH 5.0 can also cause this flocculation result. The flocculation of the particles is accompanied by a visible colour change of the suspension. This change ranges from red to violet to purple to grey and black as the particles settle from suspension. The colour change may be followed using standard techniques of analysis, e.g. the change of light adsorption by spectrophotometry.
- Sol particles can be protected from flocculation using standard techniques and protective agents known in the art. High molecular weight polyethylene glycol (i.e. 20,000 dalton molecular weight - PEG 20) or polyvinyl alcohol (10,000 to 40,000 molecular weight - PVA 10, PVA 40) have been used successfully for this purpose. In addition non-ionic surfactants, such as polyoxyethylene sorbitan monolaurate (Tween-20<sup>TM</sup>), have also been used as protectors.

- Sensitization is a phenomenon observed and described by Zsigmondy and Joel, *Z. physik. Chem.*, 113, 299 (1924). When a sufficient concentration of a polyelectrolyte like gelatin is present, a protective action occurs regardless of the charge on the gelatin molecule. When however the concentration of the gelatin is low enough, the colloidal sols are sensitized to produce the flocculation effect upon exposure to relatively lower concentrations of electrolytes. The concentration of electrolytes is insufficient to effect flocculation in the absence of the low gelatin concentration.

**Summary of the Invention**

This invention relates to the use of colloidal metal sols and to reagents and reaction mixtures comprising colloidal metal sols to quickly and easily detect the presence of thiol, mercaptan, sulfhydryl and volatile sulfur compounds in a sample. In a preferred embodiment of the invention, the sample is an exhaled breath sample from an individual suspected of having halitosis.

In one embodiment, the invention is for a method for detecting thiol or volatile sulfur compounds in a sample comprising:

- 10 (a) mixing a colloidal metal sol suspension with at least a critical protective concentration of a suitable protector to produce a protected colloidal sol suspension wherein the colloidal sol has a flocculated state which is visually distinguishable from its monodisperse suspended state;
- (b) adding a flocculating agent to the protected colloidal sol suspension in an amount sufficient to produce a tensioned colloidal sol suspension;
- 15 (c) exposing the tensioned sol to a sample suspected of containing thiol compounds,

wherein when sufficient amounts of thiol or volatile sulfur compounds are present in the sample, the tension is released and the sol flocculates in a manner to enable visual detection of the presence of thiol compounds in the sample.

- 20 In another embodiment, the invention is for a method for detecting thiol or volatile sulfur compounds in a sample comprising:

- (a) a colloidal metal sol suspension with a suitable protector, wherein the colloidal metal sol suspension has a flocculated state which is visually distinguishable from its monodisperse suspended state;

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(b) adding a polyelectrolyte to the protected sol in an amount to sensitize the sol to the presence of thiol compounds in a sample under unprotected conditions and adjusting the ionic strength of the sol to just below flocculating conditions;

5 (c) exposing the sol to a sample suspected of containing thiol compounds,

wherein when sufficient amounts of thiol compounds are present in the sample, the sol flocculates in a manner to enable visual detection of the presence of thiol compounds in the sample.

10 In another embodiment, the invention is for thiol detecting reagents comprising a tensioned colloidal metal sol suspension. The tensioned colloidal metal sol suspension comprises a colloidal metal sol suspension with at least a critical protective concentration of a suitable protector, wherein the colloidal metal sol suspension has a flocculated state visually distinguishable from its monodisperse state. The tensioned colloidal metal sol suspension further comprises a flocculating agent in an amount sufficient to place the colloidal metal sol suspension with  
15 protector in a tensioned state so that the reagent flocculates when exposed to thiol compounds.

In another embodiment, the thiol detecting reagent comprises a colloidal metal sol suspension, a suitable protector in an amount to prevent flocculation under  
20 unprotected conditions and an amount of polyelectrolyte which would normally sensitize the sol under unprotected conditions. The ionic strength of the reagent is adjusted to just below flocculating conditions. The reagent flocculates when exposed to thiol compounds, the colloidal metal sol suspension having a flocculated state visually distinguishable from its monodisperse suspended state.

25 In a preferred embodiment, the invention is for a thiol detecting kit and more preferably for a halitosis diagnostic kit comprising a sufficient amount of thiol detecting reagent and at least one blow tube, preferably one end of which can fit

into the container, the length of the blow tube preferably being longer than the length of the container.

In a preferred embodiment of the invention, the sample is a breath sample of an individual and the presence of thiol compounds in the sample is indicative of  
5     halitosis.

### **Brief Description of the Figures**

In figures which illustrate embodiments of the invention,

Figure 1 is a bar graph illustrating the clinical results of detecting volatile sulfur compounds in a breath sample of an individual using one embodiment of the  
10     method of the invention,

Figure 2 is a bar graph which illustrates the clinical results of detecting volatile sulfur compounds in a breath sample of a healthy individual using one embodiment of the method of the invention,

Figure 3 is a bar graph which illustrates the clinical results of detecting volatile sulfur compounds using an embodiment of the method of the invention in the  
15     breath sample of a patient using a series of exhales and over a number of days,

Figure 4 is a bar graph which illustrates the clinical results of detecting volatile sulfur compounds using an embodiment of the method of the invention in a breath sample of a patient before and after treatment.

**Detailed Description of the Invention**

The following is a detailed description of the preferred embodiments of the invention and is not intended to be limiting of the scope of the invention

In this specification, the use of the term thiol compounds includes thiol, mercaptan  
5 and sulphydryl containing compounds and volatile sulfur compounds thereof.

Critical protective concentration (CPC) refers to the minimum concentration of protector required to result in protecting a colloidal metal sol from flocculating under normal unprotective conditions.

Flocculation refers to a physical state of agglutination, combination, or  
10 agglomeration of suspended colloidal particles in a sol suspension.

Flocculating agent refers to a substance which induces flocculation. For instance, electrolytes or ions when added to a monodisperse sol suspension in sufficient amounts can cause flocculation.

Tension or tensioned state refers to a protected colloidal metal sol suspension  
15 containing a flocculating agent in an amount which would normally result in flocculation in an unprotected colloidal sol. The "tension" is released upon addition of minute concentrations of thiol compounds, resulting in flocculation. The thiol compounds can be either in a liquid or a gaseous state. The gases are known as volatile sulfur compounds. The release of tension results in flocculation  
20 and concomitant colour change.

Sensitization or sensitized state refers to a colloidal sol suspension which flocculates upon exposure to a lower concentration of ions or electrolytes, such as sodium chloride, calcium (II) chloride and aluminum (III) chloride) than a sol suspension in an unsensitized state. The sensitized state is actuated by adding a  
25 sensitizing amount of polyelectrolyte, such as gelatin, to a colloidal sol suspension.



The amount is less than a protective amount of polyelectrolyte. Flocculation of the sensitized sol is effected upon exposure to thiol compounds.

As stated above, in one embodiment the invention relates to a method for detecting thiol compounds in a sample comprising:

- 5 (a) mixing a colloidal metal sol suspension with at least a critical protective concentration of a suitable protector to produce a protected colloidal sol suspension wherein the colloidal sol has a flocculated state which is visually distinguishable from its monodisperse suspended state;
- 10 (b) adding a flocculating agent to the protected colloidal sol suspension in an amount sufficient to produce a tensioned colloidal sol suspension;
- (c) exposing the tensioned sol to a sample suspected of containing thiol compounds,

15 wherein when sufficient amounts of thiol compounds are present in the sample, the tension is released and the sol flocculates in a manner to enable visual detection of the presence of thiol compounds in the sample.

Preferably, the metal of the colloidal metal sol is selected from the group of metals consisting of gold, silver iodide, silver bromide, copper hydrous oxide, aluminum hydroxide or hydrous oxide, chromium hydroxide or hydrous oxide, vanadium oxide, arsenic sulfide, manganese hydroxide, lead sulfide, mercury sulfide, barium sulfate, titanium dioxide, bentonite, and clay. Most preferably, the colloidal metal sol is a colloidal gold or silver sol.

Suitable flocculating agents would be known to persons skilled in the art.

Preferably, the flocculating agent is a flocculating salt, preferably selected from the group consisting of: sodium chloride, calcium (II) chloride, and aluminum chloride.

Alternatively, the flocculating agent is preferably an acid, preferably selected from the group consisting of hydrochloric acid and citric acid, and wherein the amount of electrolyte used causes the pH of the sol to drop below 5.0.

5 In a most preferred embodiment, the flocculating agent is a citric acid salt and it is added in an amount to cause the pH of the sol to drop to between 2 to 3.5.

Although protectors suitable for practising the invention would be familiar to persons skilled in the art, preferably the protector is selected from the group of protectors consisting of: high molecular weight polyethylene glycol, polyvinyl alcohol having a molecular weight from 10,000 to 40,000, polyoxyethylene  
10 sorbitan monolaurate, gum arabic, dextran from *Leuconostoc mesenteroides* having molecular weight of about 160 daltons, alginic acid, calf skin gelatin, and pig skin gelatin.

In one embodiment of the invention, the particle size of the colloidal metal in suspension can be varied to change the amount of protector required to protect the  
15 colloidal metal sol, wherein the variation of the ratio of colloidal metal particle size and amount of protector results in a change in the detection limits for the presence of thiol compounds. The particle size of the colloidal metal used in the invention is preferably between 10 and 120 nm.

In another preferred embodiment of the invention, the concentration of the  
20 colloidal metal in the suspension can be varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of concentration and protector ratio results in a change in detection limits for the presence of thiol compounds.

In a most preferred embodiment of the invention, the colloidal metal sol is a  
25 colloidal gold sol, the colloidal gold having a particle size of between 10 to 120 nm and a concentration of between 0.01 to 0.05 weight percent

In an alternative embodiment, the invention is for a method for detecting thiol compounds in a sample comprising:

5 (a) a colloidal metal sol suspension with a suitable protector, preferably a non-ionic detergent such as Tween-20™, wherein the colloidal metal sol suspension has a flocculated state which is visually distinguishable from its monodisperse suspended state;

10 (b) adding a polyelectrolyte, preferably gelatin, and more preferably, a high molecular weight dextran, to the protected sol in sufficient amounts to sensitize the sol to the presence of thiol compounds in a sample under unprotected conditions and adjusting the ionic strength of the sol to just below flocculating conditions;

(c) exposing the sensitized sol to a sample suspected of containing thiol compounds,

15 wherein when sufficient amounts of thiol compounds are present in the sample, the sol flocculates in a manner to enable visual detection of the presence of thiol compounds in the sample.

20 In a most preferred embodiment, the methods of this invention are used to diagnose halitosis in an individual, the sample being a breath sample from the individual and the presence of the thiol compounds in the sample being indicative of halitosis.

In yet another embodiment, the invention is for a thiol detecting reagent comprising a tensioned colloidal metal sol suspension comprising a colloidal metal sol suspension with at least a critical protective concentration of a suitable protector, and a flocculating agent in an amount sufficient to produce a tensioned  
25 colloidal sol suspension and wherein the reagent flocculates when exposed to a

thiol compound. The colloidal sol has a flocculated state which is visually distinguishable from its monodisperse state

Alternatively, the thiol detecting reagent comprises a colloidal metal sol suspension with a suitable protector against flocculation and a polyelectrolyte in an amount  
5 which would sensitize the sol under unprotected conditions. The ionic strength of the reagent is adjusted to just below flocculating conditions. The colloidal metal sol suspension has a flocculated state which is visually distinguishable from its monodisperse suspended state.

In another aspect, the invention is for a halitosis diagnostic kit comprising at least  
10 one, preferably a clear, container containing a sufficient amount of thiol detecting reagent of the invention, preferably red or pink in colour. The kit also preferably comprises at least one blow tube, one end of which can fit into the container. The blow tube is preferably of greater length than the container to enable an individual to blow in one end of the tube while maintaining the other end of the tube in  
15 contact with the thiol detecting reagent.

In a preferred embodiment, the kit also comprises at least one sample of a thiol compound as a positive control and preferably instructions on how to use the kit.

In a more preferred embodiment, the blow tube of the kit is tapered at one end and preferably comprises a liquid and solid impermeable filter, the filter being  
20 permeable to gas.

Preferably, the individual grasps the blow tube at the non-tapered end avoiding touching the tapered end, and submersing the tapered end of the blow pipe into the thiol detecting reagent, and exhaling at least one time and preferably up to five times slowly and completely into the blow tube from the non-tapered end, wherein  
25 a colour change of the reagent to violet, purple, blue, gray or black indicates current halitosis. Preferably, the individual should close his/her mouth and breath

normally through the nose for approximately three minutes prior to first exhaling through the blow tube.

## EXAMPLES

### PREPARATION OF COLLOIDAL METAL SOL SUSPENSIONS

5

#### Preparation of Colloidal Gold

Preparation of 20 nanometer (nm) colloidal gold using modified method of Frens, *supra*, from Horisberger Microscopy and Rosset, *The Journal of Histochemistry and Cytochemistry* 25(4), 295-305 (1977).

#### Preparation of 0.010% (w/v) colloidal gold sol suspension

- 10 Heated 200 milliliters (ml) of distilled water to boiling in a 300 ml siliconized thick walled beaker. Added 2.0 ml of 1% (w/v) trisodium citrate dihydrate, ultrapure and 0.2 micrometers (mm) Nylon 66 membrane filtered. Continued to boil. Then added 0.5 ml of 4% (w/v) gold chloride (tetrachloroauric III acid, trihydrate, available from Sigma Chemical Co.). Boiled for five minutes. Allowed to cool. Added 40 mg of sodium azide. Particulate concentration is 0.01% w/v. Particle size is 20 nm.
- 15

#### Preparation of 0.025% (w/v) colloidal gold sol suspension

- The procedure is similar to the one used to prepare the 0.010% (w/v) colloidal gold sol suspension. 70 mg solid  $\text{HAuCl}_4$  was added to 280 ml of boiling distilled water. Added 7 ml of 0.22 micron filtered 1% (w/v) trisodium citrate. Allowed to cool. Added 60 mg sodium azide. Particulate concentration is 0.025% (w/v). Particle size is 40-50 nm and color is cherry red.
- 20

**Protection Limit of 20 nm colloidal gold preparation.**

Diluted stock 10% polyethylene glycol, 20,000 (PEG-20), sterile filtered 1:10 in distilled water. Ten microliters of the diluted stock PEG--working solution-- added to 200 microliters of colloidal gold. Serially diluted two-fold from 1:2 through  
 5 1:1024. Mixed then added 10 microliters ( $\mu$ l) of 1M HCl to the mixture.

Table I					
Determination of Critical Protective Concentration					
<u>Concentration of PEG-20% (w/v) x 10,000 [Serial Dilution of PEG-20]</u>					
	10[1:64]	5[1:128]	2.5[1:256]	1.25[1:512]	0.63[1:1024]
	CPC				
10	<u>Flocculation.</u>				
	+/-	-	-	+	+
	[PROTECTING CONCENTRATIONS]			[UNPROTECTING]	

Final Concentration of PEG-20 in cAu at critical protective concentration (CPC):  
 15 0.0005% (w/v).

Other protective agents tried:

Gum Arabic; Gum Xanthine; Dextran from *Leuconostoc mesenteroides.*, 10K, 160K, 515Kdalton molecular weights; PEG-2000; Polyvinyl alcohol 10K and 40K and Alginic acid and calf skin 225 Bloom and Pig skin 175 Bloom gelatin. Also,  
 20 Tween-20™ nonionic detergent. The CPCs, expressed as the least dilution of a 1% (w/v) solution needed for protecting colloidal gold sol for each of these protectors using the above procedure:

	<b>Table II</b>
	<b>Other Protectants (CPC in % w/v)</b>
	Gum Arabic (0.1%)
	Gum Xanthine (No protection under the conditions)
5	Dextran from <i>Leu. mes.</i> , 10K(No protection), 160K(1%), 515Kdalton (None)
	PEG-2000Kd (None)
	Polyvinyl alcohol 10K(0.01%) and 40K (0.1%)
	and Alginic acid (0.001%)
	Calf skin Gelatin (0.001%) and Pig Skin Gelatin (0.001%)
10	and Tween-20™ (0.1%).

### Preparation of PEG-20-cAu and Limit of Sensitivity

To 1 ml of 0.01% w/v PEG-20 added 20 ml of cAu and mixed by vortexing for 30 seconds. Waited 10 minutes. Added 1 ml of 1M HCl.

In a microtiter plate, added 10 microliters of dithiothreitol (DTT) at concentrations ranging from 100, 50, 25, 12.5, 6.3 mM and water as blank. Mixed 200  $\mu$ l PEG-protected gold sol to these solutions and mixed. Observed flocculation. Colour change to blue-gray indicated flocculation. Red colour indicated no flocculation. Limit of detection is the concentration of DTT in the series which flocculates the PEG-cAu in five minutes. An example of the detection limit for thiol-testing reagent is as follows. Ten microliters of the listed DTT solutions were added to 200 microliters of the colloidal gold sol-protected-acidified reagent in successive microtiter plate wells. Any flocculation was observed as a color change to violet-gray-black from red or orange and recorded. The limit of sensitivity is defined as the least amount of DTT in the serially -two fold diluted series of DTT to produce flocculation.

A stock of 10 mM DTT (MW 154) was prepared by adding 1.54 mg per milliliter of water into polypropylene cryovial sealed via a O-ring. One-to ten dilution (0.1 ml to 0.9 ml of water) of the solution was made in water to make a 100 mM working solution of DTT. This was serially diluted in cryovials by the same

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procedure as described above except that 0.5 ml of water was used as the diluent in the series ranging from 1:2, 1:4, 1:8, 1:16 making the concentrations of the working solutions 100, 50, 25, 12.5, 6.3 mM.

#### Other Reducing Agents Ineffective

- 5 In another experiment, other reducing agents such as sodium metabisulfite (sodium bisulfite) and ascorbic acid 1M each were added to a series of colloidal gold suspensions 0.2 ml of cAu reagent protected by Gum arabic, PEG-20, PVA-10 and PVA-40 at their CPCs and acidified to pH 3.0 with HCl. No reaction occurred using 20  $\mu$ l of these reducing agents, indicating that the reaction requires a
- 10 sulfhydryl group and is not just due to reduction capacity.

#### pH Effect with PEG-20 as Protector

- The pH of 20 nm colloidal gold protected by PEG-20 at the CPC is adjusted and measured using a glass pH electrode. The sensitivity of each of these reagent preparations to be flocculated by decreasing concentrations of DTT as described
- 15 herein was determined. Note that pH 6.1 did not contain added HCl and did not flocculate even with higher concentrations of DTT, indicating that the "tension" of the system was not achieved.



Table III

**The Effect of pH on the Sensitivity to Detect Thiol**

<u>pH of reagent</u>	<u>DTT Detection Limit, 10 <math>\mu</math>l per 200 <math>\mu</math>l Reagent</u>
6.1	Did not Flocculate
2.7	50 mM
2.2	50 mM
1.7	50 mM
1.4	25 mM
1.2	25 mM

**Effect of Different Particle Size Colloidal Gold  
Preparations on CPC of Polymers**

Three preparations of colloidal gold sol prepared according to Horisberger at concentrations of 0.05% (w/v) and 17, 32 and 50 nm particle sizes were prepared. Before use the preparations were diluted using distilled water for a final concentration of 0.01% (w/v). PEG-20K, PVA-10K and PVA-40K were diluted in water and the CPC for each protectant and particle size was determined as described before.

Table IV

**The Effect of Particle Size of Gold Sol on the CPC**

<u>Particle Size, nm</u>	<u>CPC, mg Polymer per 20 mg sol</u>		
	<u>PEG-20</u>	<u>PVA-10</u>	<u>PVA-40</u>
17	1.25	>50	25
32	1.25	50	50
50	1.25	12.5	50

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The results indicate that differences exist with respect to protection among polymers, between sizes of the same polymers and with the particle sizes of the sol.

#### **Effect of Particle Size on Thiol Detection Limit**

- 5     The detection limit of dithiothreitol for PEG-20 at the CPC (1.25 mg/20 mg sol) for each sol preparation was determined to be 50 mM DTT (10  $\mu$ l for 200  $\mu$ l of reagent) for 17 nm and 25 mM DTT for 32nm and 50 nm preparations. Thus particle size affects the limit of detectability of the invention.

#### **No Reaction With Gelatin Protection**

- 10    When gelatin was used as a protectant in the system, there was no reaction with thiol under the same conditions used for PEG and PVA, at the CPC using 20 nm gold sol. Thus, the type of polymer and possible the charge of the polymer has an effect upon its capability to perform as a thiol or mercaptan detector.

#### **The Effect of Electrolyte or Acid as the Flocculating agent.**

- 15    Experiments to determine whether salt can replace the low pH as a flocculating condition were performed. The use of sodium chloride solutions was tried, while the pH was unchanged at 6.1. Use of 10 ml of a saturated solution of sodium chloride was added to PEG-20 protected 20 nm colloidal gold sol. The limit of sensitivity was found to be 12.5 mM DTT, an improvement in sensitivity compared  
20    to pH 2-3 and HCl. However, there was a slow "clearing" or fading of the color of the suspension with the use of this flocculating agent compared to acidification so HCl and citric acid addition was mainly used.

Al(III) Chloride was also used and this caused both a pH decline (to 3-3.5) and a trivalent cationic effect. 10 ml of 100 mM Al(III)Cl<sub>3</sub> per 200 ml sol for flocculation action was used. The result was similar to NaCl, above, with detection limit for DTT equal to 12.5 mM.

- 5 The best result of all was with citric acid salt additions to pH 2-3.5 as this produced a stable preparation which did not fade over time. Also, the addition of Tween-20™ to the preparation at between 0.05 and 0.01% (w/v) final concentration was an improvement to prevent the accumulation of flocculated gold to plastic surfaces. Tween-20™, despite its protective properties did not affect the
- 10 detection limit of the reagent when PEG-20 was used.

#### The Effect of Varying Concentrations of Colloidal Gold on the CPC

Colloidal gold, 50 nm was used to determine the effect of particle concentration on the CPC of the protectant, PEG-20. The concentration varied from 0.0125% to 0.05% weight percent.

15

Table V	
The Effect of Gold Sol Concentration Upon CPC	
<u>Gold Sol, concentration %</u>	<u>CPC of PEG-20, mg%</u>
0.0125	6
0.025	10
0.05	25

20

The results indicate that more protectant is required to coat or protect the greater number of particles found in higher concentrated gold sols.

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## A MORE SENSITIVE METHOD FOR DETECTION OF THIOL COMPOUNDS

This procedure is a new method for detecting and measuring volatile sulfur compounds sensitively. It uses colloidal gold, high molecular weight dextran (5-40 million), and Tween-20™. This preparation was tested for the first time with 2-mercaptoethanol (2-ME) solutions.

A preparation using gelatin-sensitized colloidal gold is also prepared and used with aluminum trichloride in place of hydrochloric acid to detect visually the presence of volatile and soluble thiol compounds.

### Sensitization I: High MW Dextran

Prepared 1 mg/ml 5-40 million molecular weight dextran by dissolving at room temperature in PBS/50 and filtering the cloudy suspension through 0.2  $\mu$  nylon filter.

Prepared dilutions in PBS/50. One tenth ml per 900 microliters of colloidal gold. Added neat, 1:10, 1:100, 1:1000 dilutions of dextran and then added gold. The 1:100 dilution flocculated immediately with a violet color. This is sensitization.

Table VIII  
Dilution of 1mg/ml dextran

	<u>0</u>	<u>1:10</u>	<u>1:100</u>	<u>1:1000</u>	<u>Infinity [blank]</u>
Concentration, final	0.1	10ug/ml	1 ug/ml	0.1ug/ml	0
Flocculation +/-	-	-	+	-	-
Sensitization					

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Prevention of Sensitization with Tween-20, a Protectant.

Added 10  $\mu$ l of 0.1% Tween-20<sup>TM</sup> in water to 0.9 ml colloidal gold. Final concentration of Tween-20 is 0.001% (w/v) Tween-20. Then added 0.1 ml of 1:100 dilution (10  $\mu$ g/ml dextran) to this. The nonionic detergent prevented or  
 5 protected the colloid from sensitization. Then acidified by adding 10  $\mu$ l of 1M HCl = 0.01M HCl, pH was around 2.0.

**Assay of 2-ME Dilutions for Detection Limit.**

Prepared 5 ml of the above preparation in glass. Some adsorption of the cAu occurred to the glass surface.

10 Added 0.2 ml of thiol-detecting preparation to 10  $\mu$ l of 0.1mM 2-mercaptoethanol diluted 500 fold. All flocculated. 0 control (blank) did not.

15

Table IX											
<i>Dilution of 0.1mM 2-ME</i>											
	0	2	4	8	16	32	64	128	250	500	Infinity (Blank)
Flocculation											
+/-	+	+	+	+	+	+	+	+	+	+	-

10  $\mu$ l per 200  $\mu$ l prep.

Preparation of 2-ME. Added 10.2 mg of the thiol in 1.3 ml water (.1M) then  
 20 diluted this 1:1000 (0.1mM) for stock.

Detection limit for 2-ME is 2 picomole (pmol).

The invention is sensitive to a thiol (2-mercaptoethanol) and a dithiol (dithiothreitol).

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### Sensitization II: Gelatin

The use of dilute concentrations of gelatin to produce sensitization of a colloidal sol is well known. To prepare a thiol-indicating reagent, colloidal gold was first "protected" slightly by adding the non-ionic detergent Tween-20, then a solution of gelatin which proved to be sensitizing in the absence of said protection is introduced into the colloidal gold-Tween-20 suspension. A solution of aluminum chloride was added to a concentration range which was slightly less than that necessary to flocculate the mixture. Results of studies using this preparation indicated a very sensitive reagent to added volumes of soluble and gaseous thiols, with an improved color formation--tending to be more distinctly gray-black when positive for VSCs.

### Determination of Sensitization by Gelatin

Calf skin Type III Gelatin: 225 Bloom Units, Sigma was prepared in TBS at a 1% (w/v) concentration. The grains were allowed to swell for 10 minutes, then the 10 ml of liquid heated for 15 seconds in a microwave oven to dissolve.

A 1:10 and 1:100 dilution of this was prepared in distilled water, then 1:200, 1:300 and 1:400 dilutions further prepared.

10 microliters of these were added followed by 200 microliters of the bare 0.025% (w/v) colloidal gold suspension. Any immediate flocculation observed is a sensitization by gelatin.

5

Table X						
RESULTS						
Sensitization of cAu by Gelatin						
Dilution of 1% gelatin						
	100	200	300	400	1000	00
Flocculation	none	none	yes	yes	none	none

10

Thus, 10 ul of 1:400 dilution of gelatin sensitized 0.025% (w/v) cAu, or 0.25 ug (250 ng of gelatin is needed to sensitize 50 ug of cAu. 5 ug of gelatin sensitizes 1 mg of colloidal gold).

15

Table XI		
Preparation of Gelatin-cAu Thiol Indicator.		
<u>Component</u>	<u>Concentration</u>	<u>Volume</u>
Colloidal gold	0.0255% (w/v)	20 ml
Tween-20	1% (w/v)	0.2ml
Calf skin Gelatin	1/400% (w/v)	1 ml
AlCl <sub>3</sub>	6.25mM	1 ml

### Testing

20

The above preparation was tested using aqueous dissolved 2-mercaptoethanol and a laboratory technique for producing volatile thiols.



Table XII					
Detection Limit: Dissolved 2-ME.					
	<u>100uM</u>	<u>50 uM</u>	<u>25 uM</u>	<u>12.5 uM</u>	<u>0</u>
Flocculation					
5 +/-	++	+	-	-	-

Added 10 microliters of 2-ME to 200 ul of gelatin-sensitized reagent.

Detection limit is 0.5 nmol of 2-ME.

**SILVER SOLS FOR COLORIMETRIC DETECTION OF MERCAPTAN**

Preparation: Silver sol was prepared from silver nitrate and reduced by hydrazine hydrate. Solid silver nitrate was prepared in a 2% (w/v) aqueous stock. This was diluted 1:100, 1:200 and 1:300 with water (final concentrations: 0.02% (w/v);

- 5 0.01% (w/v) and 0.0067% (w/v), respectively). One milliliter of these working dilutions was added to 50 microliters of a 1 milliliter per liter solution of hydrazine hydrate, 85% (w/v), equivalent to 55% (w/v) hydrazine). This is equivalent to adding 27.5 nanograms of hydrazine. The 0.02% preparation produced an orange sol; 0.01% produced green and the 0.0067% was pink colored. All were cloudy.
- 10 These solutions were tested using added liquid additions and the proportions of 2 mercaptoethanol as described before.

15

Table XV						
Detection Limit Titration						
<u>2-mercaptoethanol, mM</u>						
Silver Concentration	<u>100</u>	<u>50</u>	<u>25</u>	<u>12.5</u>	<u>6.3</u>	<u>0 mM</u>
	Flocculation					
0.02% (w/v)	+	+	+	+	+	-
0.01% (w/v)	+	+	+	+	+	-
0.0067% (w/v)	+	+	+	+	+	-

- 20 The limit of detection for all silver preparations is 6.3 mM thiol.

## PROCEDURE AND METHODS FOR DETECTING ORAL MALODOR

### Clinical Testing

A preparation of thiol-detecting reagent was prepared and used to determine the presence of volatile sulfur compounds--mercaptans--in the exhaled breath of individuals who have oral malodor. The method described herein is novel in that one can detect these compounds by self-assessment, by blowing in a tube filled with the thiol-detecting reagent, the subject of this disclosure, followed by a visual examination of a color change if the compound(s) is/are present in the exhaled breath.

10

### Tension Method for Detecting Oral Malodor

A PEG-20 protected colloidal gold sol, 20 nm and 0.02% (w/v) concentration with Tween-20™ and pH of 3.5, was placed into either 12 x 75 mm polystyrene test tubes or 1.5 ml capacity microcuvettes. A volume of 0.5 ml was used throughout. The subjects were allowed to exhale into a tapered polypropylene breathing tube which was placed within the red liquid. The subject was instructed to blow or exhale breath into the tube, bubbling the liquid for 1, 2, 3, 4, 5 and 20 repetitions into a triplicate series of reagent-containing tubes. This occurred first morning without brushing or any other oral hygiene. After an incubation period that ranged from 2-30 minutes, distilled water, 0.5ml was added to all resulting solutions, and the color of the sol was determined by reading the absorbance of the light at 525 nm in the 1 cm path length cuvette. The results for seven determinations over a 26 day period for someone with chronic oral malodor and periodic determinations with someone without the condition were assessed. See Figures 1-4 bar graphs which illustrate the results of this test.

The results of the study, as illustrated in Figures 1 and 3, showed that a patient with oral malodor produced a colorimetric response when the expired air was

captured and reacted with the thiol-reacting reagent. Furthermore, the reaction appeared to be semi-quantitative or exhibiting a dose-responsive pattern: more replicate breaths produced a greater color change on average. There was a great degree of variability with the results but this is not unexpected given the  
5 complexity of liquid-gas volatilization process from the mouth by expiration alone.

Referring to Figure 2, the effect of the thiol detecting test with an individual without oral malodor was different than with an individual malodor. Very few breaths produced a color change.

Figure 4 illustrates that treatment of the oral malodor patient with common over-the-counter tooth paste, flossing and an application of a 3% hydrogen peroxide  
10 gargle did not lessen appreciably the amount of color response shown with the test. This indicates that there might be a pool of thiol compounds in the mouth tissues that are not reached by these agents and are continued to be volatilized and reactive to the thiol-detecting reagent described herein. It also indicates that  
15 development of a better treatment for halitosis is needed.

In summary, the results of the clinical trials indicate that the thiol-detecting reagent is capable of detecting the minute concentrations of thiol-volatile compounds released by and are the causative agent of the oral malodor condition. There is no response with a patient with no oral malodor. A treatment with brushing, flossing  
20 and hydrogen peroxide gargle did not diminish the response and color indicating result of the detection.

### **Sensitization Method for Detecting Oral Malodor**

Sixty patients who presented themselves to a dental clinical at the University of Michigan, Ann Arbor were tested prior to examination and any treatment using a  
25 Tween-20-protected colloidal gold thiol detecting device having a detection limit of 1 nmole DTT/200ul. One-half milliliters of reagent placed in a 12 x 75 mm

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polystyrene culture tube was subjected to one minute of exhaled breath for each individual blowing through a pipette tip inserted into the fluid. The absorbance value at 540 nm was measured for each reactant.

5 Results: The results of the study showed that two (2) of sixty tested individuals possessed sufficient thiol to flocculate the colloidal gold. Flocculation was detected by a decrease in the absorbance.

#### Testing: Volatilizer

10 A filter paper disk with added solution of 10 mM 2-ME was inserted on top of a aerosol plugged 1000 ul blue pipette tip. A pipump 10ml volume pipette pump was used to flow a discrete and metered amount of air through this filter disk and volatilize the thiol. The number or volume of air required to produce a flocculation of 0.5 ml of gelatin-thiol reagent is a measure of the detection limit for volatile sulfur compounds, since liquid droplets are not expected to travel through the hydrophobic plug.

Table XIII				
Volatilizer results				
Number of Replicate Pumps	Total Volume, ml	Absorbance @540nm	Abs Change	
0	0	0.641	---	
1	10	0.649	0.008	
2	20	0.642	0.001	
3	30	0.643	0.002	
4	40	0.647	0.006	
5	50	0.666	0.025	
8	80	0.489	-0.152	
10	100	0.514	-0.127	

#### Testing by the Halimeter RH-17.

The Halimeter is a commercially available device which purports to detect volatile sulfur compounds in the expired air of patients. The subject breathes into a straw and a volume of mouth air is swept by a pump at 1.5 SCPH into a patented device (US Patent No. 4,017,373) and registered on a number scale. There is some background readings and the operations manual indicate that a peak value of "300" is a barely detectable oral malodor score.

Using the same 10 mM 2-ME used in the thiol detection device above, the following information was obtained using the Halimeter.

Table XIV		
<u>Halimeter Reading, Peak</u>	<u>CODE</u>	<u>Organoleptic</u>
71.8 +/- 5.5	Distilled water, pipette tip	
113.5 +/- 11	10 mM 2ME in filter paper	3, mildly offensive

- 5 Note that the Halimeter did not register values of over 300 with the amount of 2-ME that generated a positive response with the subject of this patent disclosure and also with an organoleptic determination by a trained dentist of "3" mildly offensive" for the pipette-filter paper.

10 Thus it is apparent from this research that the subject of the invention is better able to detect volatile sulfur compounds than the commercially available instrument.

## KITS

### Contents and Directions: One Illustrative Example

Contents: One 12 x 75 mm clear polystyrene culture (test) tube with fitted cap. Contains 0.5 to 1.0 milliliter of bad breath detection reagent which is preferably  
15 red or pink colored. A tapered hollow pipe serves as a blow pipe through which one exhales expired breath, bubbling this into the liquid bad breath reagent.

Directions: Close mouth and breathe normally inspiring and expiring through the nose for approximately three (3) minutes.

20 Pull out cap from tube and set aside. Grasp tapered blow pipe of bad breath test unit at the finned section. Avoid touching smooth tapered section of pipe with fingers and insert blow pipe into test tube with the point of the tapered end at the bottom an of the tube submerged into the red reagent. Press lips around open blow pipe and exhale, slowly and completely. There will be some back pressure. The

red liquid will bubble up but not out of the tube. Repeat exhaling in succession four more times, a total of five.

Observe color of liquid. A paler shade of red--to pink--is observed sometimes and is negative. Any color change to violet, purple, blue, gray or black indicates  
5 current breath malodor. Withdraw and discard pipe and recap tube. The amount of VSC can be determined by measuring the light absorbency at 595 nm of the reacted liquid.

In general, it will be apparent to one skilled in the field of such tests that the desirable commercial embodiments of the present invention include the provision  
10 of a pre-packaged kit of materials for detecting thiol mercaptan sulfhydryl or volatile sulfur compounds in expired breath by the methods set forth above. Such a kit would comprise for example, a reagent consisting essentially of a colloidal agent that has a flocculated state which is visually distinguishable from its monodisperse suspended state, the colloidal agent having adsorbed onto its surface  
15 a protective amount of a polymer or nonionic detergent, and an additional reagent consisting of an aqueous solution of an acid or a base electrolyte. Desirably, there would also be included a positive control reagent comprising a sample of a sulfhydryl-or mecaptan- or thiol-containing compound, and one or more implements useful in performing said test, e.g., a test tube for holding and  
20 observing the state of the colloidal sol, and a tube or pipe or other conduit which is inserted into the test tube and said reagent, through which expired air is introduced. A barrier to liquid or solid but freely permeable to gas, contained within this said tube, pipe or conduit, such as for general examples, filter paper, porous membrane or glass or cotton fibers is optionally situated for the purpose of preventing  
25 unintended materials emanating from the act of sampling, e.g., saliva, or plaque or mucous, or food debris or other potentially contaminating material of the oral cavity.

For the purpose of use of the describe kits, an individual suspected of possessing the oral malodor condition is allowed to breathe or expirate spent breath or exhale

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from the mouth through the aforementioned tube, pipe or conduit. The flow of the gas as a result of the exhalation, forces the gas to mix and bubble through the liquid reagent describe herein contained within the test tube in the said kit. The bubbling action of the gaseous sample throughout the liquid reagent causes a  
5 dissolving into the liquid reagent of any gas or volatile compound in the sample.

Any thiol containing compound which is volatilized from the oral cavity of the individual subjected to the test will dissolve into the reagent. A reaction will take place which produces a destabilization of the protected colloid. Subsequently the colloid flocculates. This flocculation is visually distinct from the colloidal sol  
10 state.

In such a kit, the colloidal agent is desirably selected from a the group consisting of: gold, silver iodide, silver bromide, copper hydrous oxide, aluminum hydroxide or hydrous oxide, chromium hydroxide or hydrous oxide, vanadium oxide, arsenic sulfide, manganese hydroxide, lead sulfide, mercury sulfide, barium sulfate,  
15 titanium dioxide, bentonite, and clay.

The thiol-compound is optionally a member of the group that includes: hydrogen sulfide, methyl mercaptan, dimethyl mercaptan, dithiothreitol, 2-mercaptoethanol, cysteine, glutathione( reduced form), mercaptoacetic acid (a.k.a. thioglycolic acid), thiosaliclic acid, 2-mercaptoethanesulfonic acid, 2-mercaptoethylamine, 2-  
20 mercaptoimidazole, 2-mercapto-5-nitrobenzimidazole, 3-mercapto-1,2-propanediol, thiolactic acid, 3-mercaptopropionic acid and other related compounds.

It is understood that the invention is not limited to the illustrations described and shown herein, which are merely illustrative of the best modes of carrying out the invention, and which are suitable of modification of form, size, arrangement of  
25 steps and details of operation. The invention rather is intended to encompass all such modifications which are within the spirit and scope of the present disclosure, and is defined by the appended claims.

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**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE  
PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. A method for diagnosing halitosis in an individual comprising:

5 (a) mixing a colloidal metal sol suspension with at least a critical protective concentration of a suitable protector to produce a protected colloidal sol suspension wherein the colloidal sol has a flocculated state which is visually distinguishable from its monodisperse suspended state;

(b) adding a flocculating agent to the protected colloidal sol suspension in an amount sufficient to produce a tensioned colloidal sol suspension;

10 (c) exposing the tensioned sol to a breath sample of the individual,

wherein when sufficient amounts of thiol compounds are present in the sample, the tension is released and the sol flocculates in a manner to enable visual detection of the presence of thiol compounds in the sample, indicative of halitosis.

15 2. The method of claim 1 wherein the metal of the colloidal metal sol is selected from the group of metals consisting of gold, silver iodide, silver bromide, copper hydrous oxide, aluminum hydroxide or hydrous oxide, chromium hydroxide or hydrous oxide, vanadium oxide, arsenic sulfide, manganese hydroxide, lead sulfide, mercury sulfide, barium sulfate, titanium dioxide, bentonite, and clay.

20 3. The method of claim 1 wherein the flocculating agent is a flocculating salt selected from the group consisting of: sodium chloride, calcium (II) chloride, and aluminum chloride.

4. The method of claim 1 wherein the flocculating agent is an acid selected from the group consisting of hydrochloric acid and citric acid, and wherein the amount of electrolyte used causes the pH of the sol to drop below 5.0.

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5. The method of claim 1 wherein the flocculating agent is a citric acid salt and it is added in an amount to cause the pH of the sol to drop to between 2 to 3.5.
6. The method of claim 1 wherein the protector is selected from the group of protectors consisting of: high molecular weight polyethylene glycol, polyvinyl alcohol having a molecular weight from 10,000 to 40,000, polyoxyethylene sorbitan monolaurate, gum arabic, alginic acid, calf skin gelatin, and pig skin gelatin.
7. The method of claim 1 wherein the particle size of the colloidal metal in suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of the ratio of colloidal metal particle size and amount of protector results in a change in the detection limits for the presence of thiol compounds.
8. The method of claim 7 wherein the particle size of the colloidal metal is between 10 and 120 nm.
9. The method of claim 1 wherein the concentration of the colloidal metal in the suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of concentration and protector ratio results in a change in detection limits for the presence of thiol compounds.
10. The method of claim 1 wherein the colloidal metal sol is a colloidal gold sol, the colloidal gold having a particle size of between 10 to 120 nm and a concentration of between 0.01 to 0.05% weight percent.
11. A method for diagnosing halitosis in an individual comprising:
- (a) a colloidal metal sol suspension with a suitable protector, wherein the colloidal metal sol suspension has a flocculated state which is visually distinguishable from its monodisperse suspended state;

(b) adding a polyelectrolyte to the protected sol in amounts that would sensitize the sol to the presence of thiol compounds in a breath sample under unprotected conditions and adjusting the ionic strength of the sol to just below flocculating conditions;

5 (c) exposing the sol to a breath sample of an individual,

wherein when sufficient amounts of thiol compounds are present in the sample, the sol flocculates in a manner to enable visual detection of the presence of thiol compounds in the sample, indicative of halitosis.

12. The method of claim 13 wherein the protector is a non-ionic detergent.

10 13. The method of claim 13 wherein the polyelectrolyte is selected from the group consisting of gelatin, high molecular weight dextran.

14. The method of claim 13 wherein the metal of the colloidal metal sol is selected from the group of metals consisting of gold, silver iodide, silver bromide, copper hydroxide, aluminum hydroxide or hydroxide, chromium hydroxide or  
15 hydroxide, vanadium oxide, arsenic sulfide, manganese hydroxide, lead sulfide, mercury sulfide, barium sulfate, titanium dioxide, bentonite, and clay.

15. The method of claim 11 wherein the particle size of the colloidal metal in suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of the ratio of colloidal metal particle size  
20 and amount of protector results in a change in the detection limits for the presence of thiol or volatile sulfur compounds.

16. The method of claim 11 wherein the particle size of the colloidal metal is between 10 and 120 nm.

17. The method of claim 11 wherein the concentration of the colloidal metal in the suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of concentration and protector ratio results in a change in detection limits for the presence of thiol compounds.
- 5 18. The method of claim 13 wherein the colloidal metal sol is a colloidal gold sol, the colloidal gold having a particle size of between 10 to 120 nm and a concentration of between 0.01 to 0.05% weight percent.
- 10 19. A thiol detecting reagent comprising a tensioned colloidal metal sol suspension, the tensioned colloidal metal sol suspension comprising a colloidal metal sol suspension with at least a critical protective concentration of a suitable protector and a flocculating agent in an amount sufficient to cause the sol to flocculate when unprotected and wherein the reagent flocculates when exposed to a thiol compound, the colloidal sol having a flocculated state which is visually distinguishable from its monodisperse state.
- 15 20. The reagent of claim 19 wherein the metal of the colloidal metal sol is selected from the group of metals consisting of gold, silver iodide, silver bromide, copper hydrous oxide, aluminum hydroxide or hydrous oxide, chromium hydroxide or hydrous oxide, vanadium oxide, arsenic sulfide, manganese hydroxide, lead sulfide, mercury sulfide, barium sulfate, titanium dioxide, bentonite, and clay.
- 20 21. The reagent of claim 19 wherein the flocculating agent is a flocculating salt selected from the group consisting of: sodium chloride, calcium (II) chloride, and aluminum chloride.
- 25 22. The reagent of claim 19 wherein the flocculating agent is an acid selected from the group consisting of hydrochloric acid and citric acid, and wherein the amount of electrolyte used causes the pH of the sol to drop below 5.0.

23. The reagent of claim 19 wherein the flocculating agent is a citric acid salt and it is added in an amount to cause the pH of the sol to drop to between 2 to 3.5.
24. The reagent of claim 19 wherein the protector is selected from the group of protectors consisting of: high molecular weight polyethylene glycol, polyvinyl alcohol having a molecular weight from 10,000 to 40,000), polyoxyethylene sorbitan monolaurate, gum arabic, alginic acid, calf skin gelatin, and pig skin gelatin.
25. The reagent of claim 19 wherein the particle size of the colloidal metal in suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of the ratio of colloidal metal particle size and amount of protector results in a change in the detection limits for the presence of thiol or volatile sulfur compounds.
26. The reagent of claim 19 wherein the particle size of the colloidal metal is between 10 and 120 nm.
27. The reagent of claim 19 wherein the concentration of the colloidal metal in the suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of concentration and protector ratio results in a change in detection limits for the presence of thiol compounds.
28. The reagent of claim 19 wherein the colloidal metal sol is a colloidal gold sol, the colloidal gold having a particle size of between 10 to 120 nm and a concentration of between 0.01 to 0.05% weight percent.
29. A thiol detecting reagent comprising a colloidal metal sol suspension with a suitable protector against flocculation and a polyelectrolyte in an amount which would sensitize the sol when unprotected, the ionic strength of the sol adjusted to just below flocculating conditions, wherein the reagent flocculates when exposed to

thiol compound, the colloidal metal sol suspension having a flocculated sol which is visually distinguishable from its monodisperse suspended state.

30. The reagent of claim 29 wherein the protector is a non-ionic detergent.

5 31. The reagent of claim 29 wherein the polyelectrolyte is selected from the group consisting of gelatin, high molecular weight dextran.

32. The reagent of claim 29 wherein the metal of the colloidal metal sol is selected from the group of metals consisting of gold, silver iodide, silver bromide, copper hydrous oxide, aluminum hydroxide or hydrous oxide, chromium hydroxide or hydrous oxide, vanadium oxide, arsenic sulfide, manganese hydroxide, lead sulfide,  
10 mercury sulfide, barium sulfate, titanium dioxide, bentonite, and clay.

33. The reagent of claim 29 wherein the particle size of the colloidal metal in suspension is varied to change the amount of protector required to protect the colloidal metal sol, wherein the variation of the ratio of colloidal metal particle size and amount of protector results in a change in the detection limits for the presence  
15 of thiol or volatile sulfur compounds.

34. The reagent of claim 29 wherein the particle size of the colloidal metal is between 10 and 120 nm.

35. The reagent of claim 29 wherein the concentration of the colloidal metal in the suspension is varied to change the amount of protector required to protect the  
20 colloidal metal sol, wherein the variation of concentration and protector ratio results in a change in detection limits for the presence of thiol compounds.

36. The reagent of claim 29 wherein the colloidal metal sol is a colloidal gold sol, the colloidal gold having a particle size of between 10 to 120 nm and a concentration of between 0.01 to 0.05% weight percent.

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37. A halitosis diagnostic kit comprising at least one container comprising a sufficient amount of thiol detecting reagent and at least one blow tube which can fit into the container and is of greater length than the container.
- 5 38. A halitosis diagnostic kit comprising, at least one clear container, the container comprising a sufficient amount of thiol detecting reagent and at least one blow tube which can fit into the container and is of greater length than the container, wherein the reagent is a reagent according to any of claims 19 or 29.
39. A halitosis diagnostic kit of claim 38 further comprising at least one sample of a thiol compound as a positive control.
- 10 40. A halitosis diagnostic kit of claim 38 further comprising instructions on how to use the kit.
41. A halitosis diagnostic kit of claim 38, wherein the blow tube comprises a liquid and solid impermeable filter which is freely permeable to gas.
- 15 42. A kit for diagnosing halitosis comprising at least one clear polystyrene container containing 0.5 to 1 millilitre of thiol detecting reagent according to claims 19 or 29 which is red or pink coloured, at least one tapered hollow blow tube through which one exhales expired breath, bubbling it into the liquid thiol detecting reagent.
43. A method of diagnosing halitosis in an individual comprising:
- 20 (a) at least one clear tube containing about 0.5 to 1.0 millimeters of a red or pink thiol detecting reagent according to claims 19 or 29.
- (b) at least one hollow tube tapered at one end, the tapered end of which can be placed into the reagent.



wherein the individual grasps the blow tube at the non-tapered end avoiding touching the tapered end, and submersing the tapered end of the blow pipe into the thiol detecting reagent, and exhaling at least one time slowly and completely into the blow tube from the non-tapered end, wherein a colour change of the reagent to  
5 violet, purple, blue, gray or black indicates current halitosis.

44. The method of claim 43 wherein the individual first closes his/her mouth and breaths through the nose for about three minutes prior to the first exhale through the blow tube.

45. The method of claim 43 wherein the individual exhales through the blow tube  
10 five times.

46. The use of a colloidal metal sol suspension in diagnosing halitosis in an individual.

FIGURE 1/4

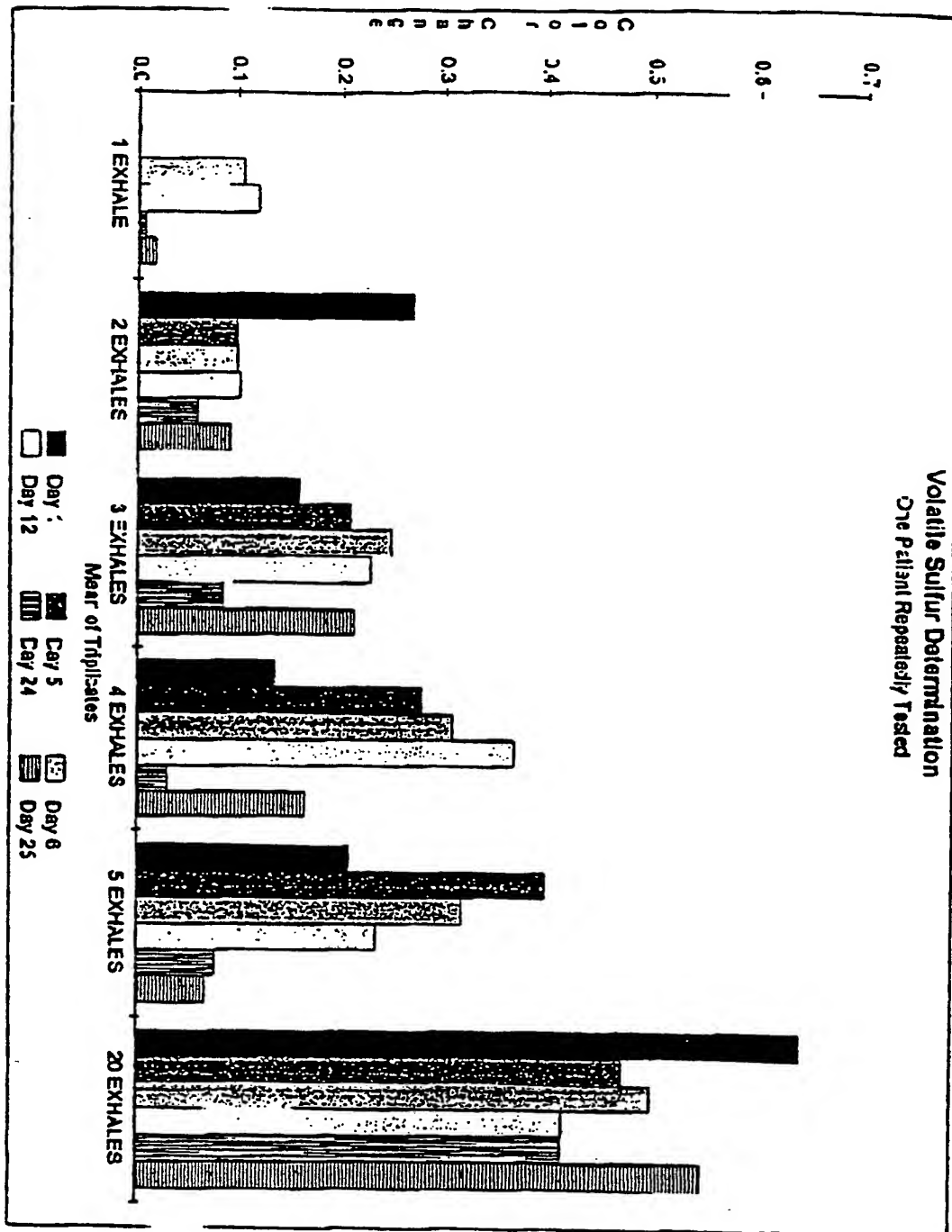
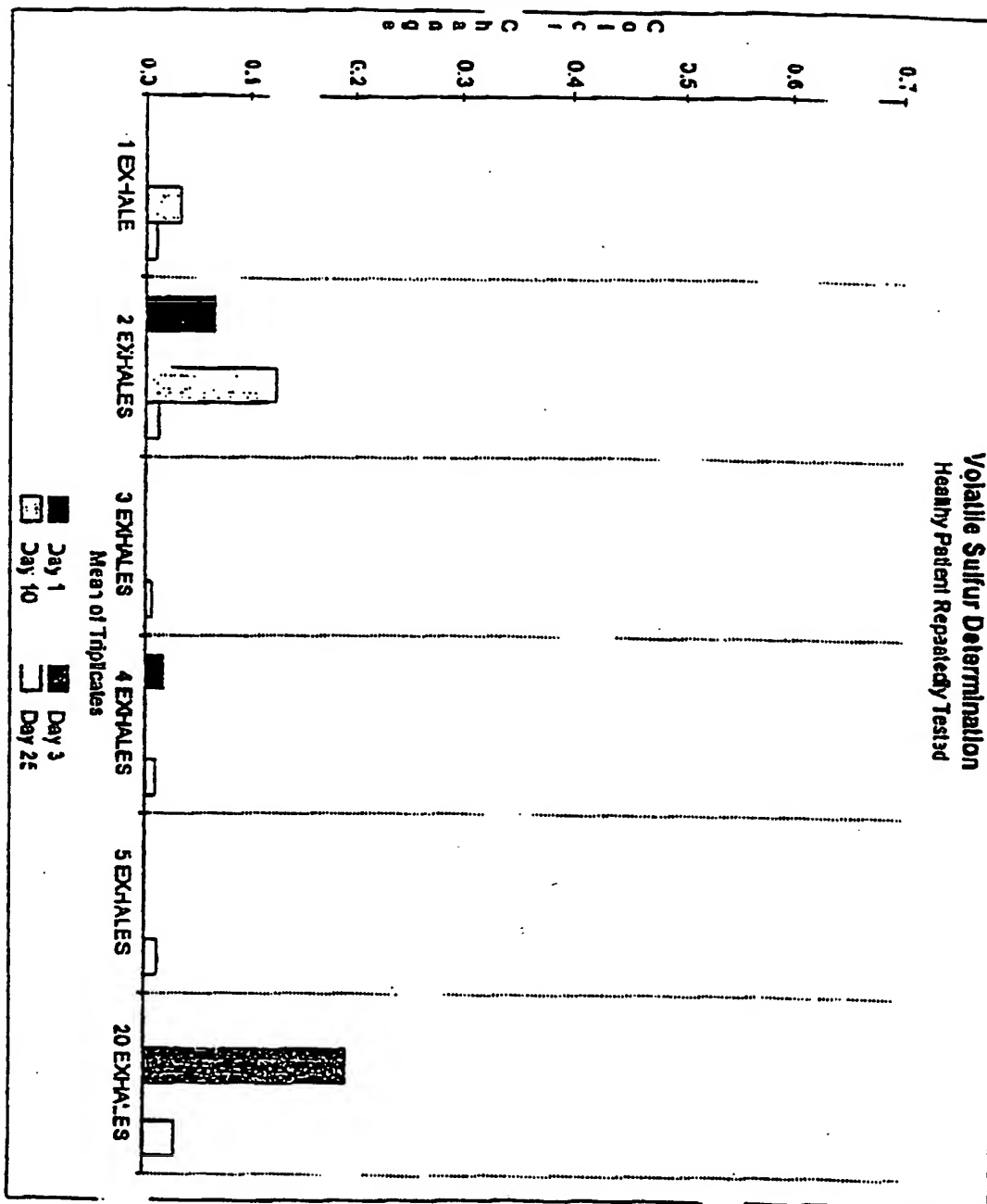
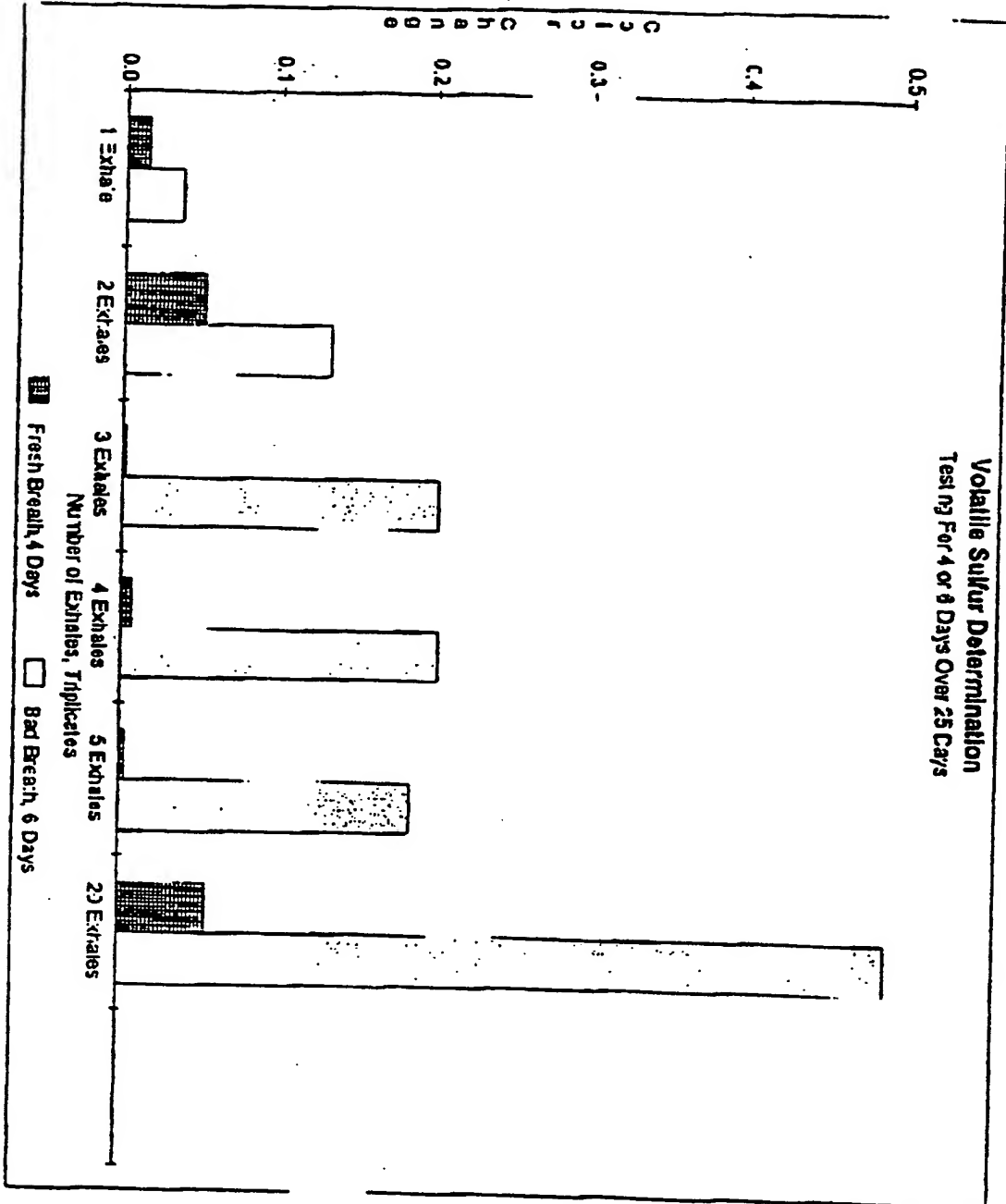


FIGURE 2/4



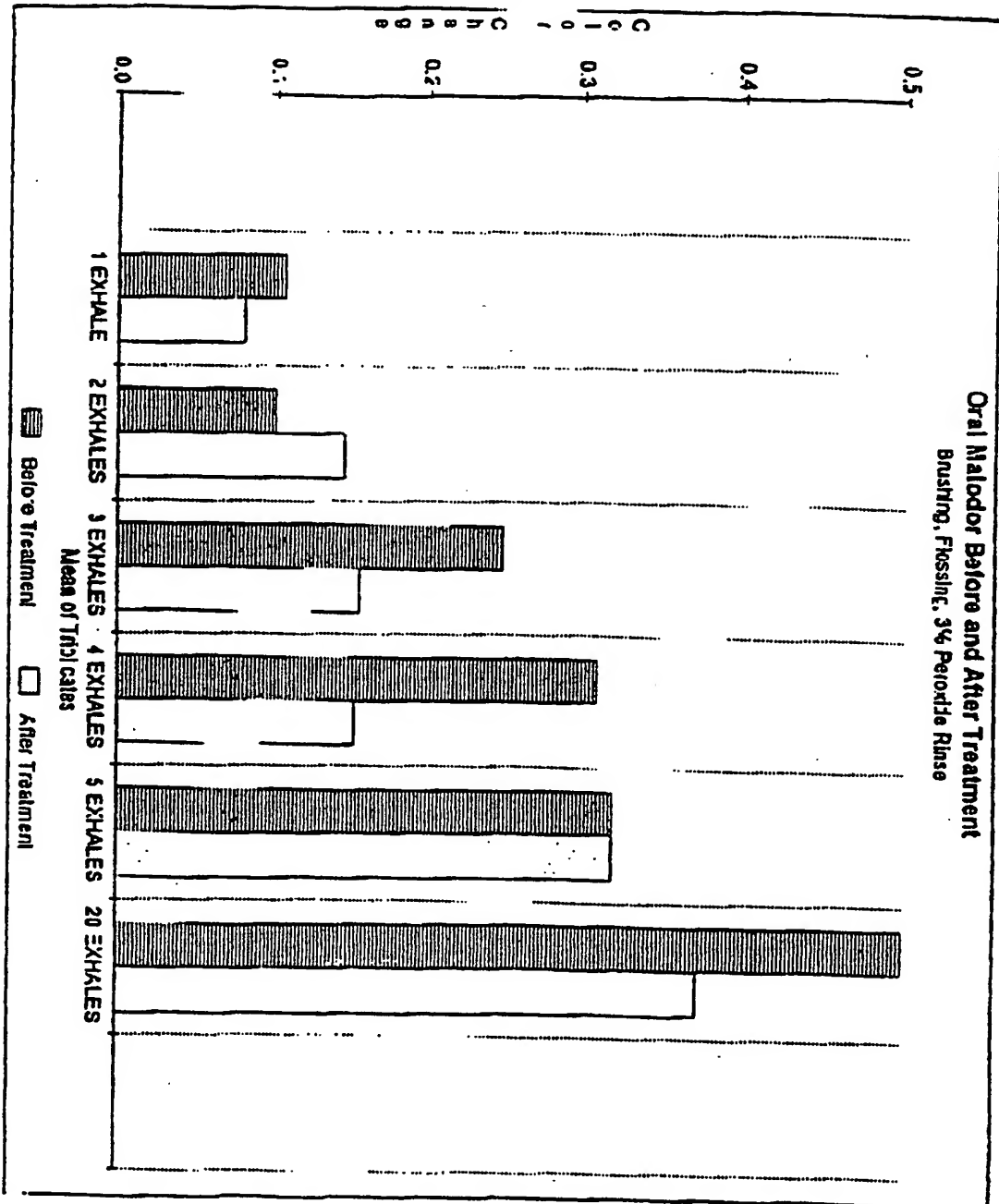
BEST AVAILABLE COPY

FIGURE 3/4



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FIGURE 4/4



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/12488

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/00  
US CL : 73/23.3; 422/83, 84,85, 61; 436/119, 120  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 73/23.3; 422/83, 84,85, 61; 436/119, 120

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS search terms: colloid?; halitos? or breath? or odor?; sulfur? or sulfhydryl or mercapt## or thiol

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A -- Y	US, A, 3,622,277 (NOLL ET AL) 23 November 1971 (23.11.71), see entire document.	1-45 ---- 46
A -- Y	US, A, 5,187,105 (ALBARELLA ET AL) 16 February 1993 (16.02.93), see column 3, lines 22-26.	1-36, 38-45 ----- 37, 46
Y	US, A, 5,310,647 (KERSCHENSTEINER) 10 May 1994 (10.05.94), see column 2, lines 5-22; column 11, lines 66-68; and column 14, lines 2-3.	19-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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